

Sensitivity of winter phytoplankton communities from Andean lakes to artificial ultraviolet-B radiation

Sensibilidad de comunidades fitoplanctónicas invernales de lagos andinos a la radiación ultravioleta-B artificial

E. WALTER HELBLING^{1,2}, VIRGINIA E. VILLAFañE^{1,2} & ELENA S. BARBIERI^{1,3}

¹Estación de Fotobiología Playa Unión, Casilla de Correo 153, 9100, Trelew, Chubut, Argentina,
e-mail: whelbling@efpu.com.ar

²Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

³Universidad Nacional de la Patagonia, Puerto Madryn, Chubut, Argentina

ABSTRACT

During July of 1999 sampling was carried out in five Andean lakes to determine the sensitivity of winter phytoplankton communities to ultraviolet-B radiation (UV-B, 280-320 nm). The studied lakes, Moreno, El Trébol, Nahuel Huapi, Gutiérrez, and Morenito, located in the Patagonia region (41° S, 71° W, 800 m of altitude), had attenuation coefficients for UV-B that ranged from 0.36 m⁻¹ (Lake Moreno) to 2.8 m⁻¹ (Lake Morenito). The samples were inoculated with labeled carbon (NaH¹⁴CO₃) and incubated in an illuminated chamber (UV-B = 0.35 W m⁻², UV-A [320-400 nm] = 1.1 W m⁻², and PAR [400-700 nm] = 10.8 W m⁻²) at 10 °C. The phytoplankton cells were exposed to UV radiation (280-400 nm) + PAR (quartz tubes), and to UV-A + PAR (quartz tubes covered with Mylar-D). The total duration of the experiments was 4 h and two samples were taken from each treatment every hour. In lakes Moreno, El Trébol, Nahuel Huapi and Gutiérrez, the photosynthetic inhibition increased linearly with UV-B doses, while in Lake Morenito just a slight relationship was observed. After receiving a dose of 1.25 kJ m⁻² (UV-B), phytoplankton from Lake Morenito had the highest cumulative photosynthetic inhibition (44 %), whereas in Lakes Moreno, El Trébol, Nahuel Huapi and Gutiérrez the inhibition was of 22, 11, 5, and 1 %, respectively. However, at the end of incubation period and after receiving doses of 5 kJ m⁻², the most inhibited phytoplankton cells were from Lake Moreno (70 %) and the most resistant (27 %) was that from Lake Gutiérrez. The kinetics of inhibition was different in each lake, and transparent lakes, with higher proportion of large cells, had higher inhibition rates. The results suggest that an increase in UV-B radiation (e.g., produced by a decrease in stratospheric ozone) would have a greater impact on microplankton from clear lakes, while pico- and nanoplankton from less transparent lakes will be less affected.

Key words: ultraviolet-B radiation, photosynthesis, phytoplankton, ozone, Andean lakes.

RESUMEN

Durante julio de 1999 se realizaron muestreos en cinco lagos Andinos con el objetivo de determinar la sensibilidad de comunidades fitoplanctónicas invernales a la radiación ultravioleta-B (UV-B, 280-320 nm). Los lagos estudiados, Moreno, El Trébol, Nahuel Huapi, Gutiérrez y Morenito, están ubicados la región Patagónica (41° S, 71° W, 800 m de altitud) y tienen coeficientes de atenuación variables entre 0,36 m⁻¹ (Lago Moreno) y 2,8 m⁻¹ (Lago Morenito). Las muestras se inocularon con carbono marcado (NaH¹⁴CO₃) y se incubaron en una cámara iluminada (UV-B = 0,35 W m⁻², UV-A [320-400 nm] = 1,1 W m⁻², y PAR [400-700 nm] = 10,8 W m⁻²) a 10 °C. El fitoplancton se expuso a PAR (radiación fotosintéticamente activa) + radiación UV (tubos de cuarzo), y a PAR + UV-A (tubos de cuarzo recubiertos con Mylar-D). La duración total de los experimentos fue de 4 h y se retiraron dos muestras de cada tratamiento cada 1 h. En los Lagos Moreno, El Trébol, Nahuel Huapi y Gutiérrez la inhibición fotosintética aumentó en forma lineal con la dosis de UV-B, mientras que en el Lago Morenito esta relación fue débil. Después de recibir una dosis de 1,25 kJ m⁻² (UV-B), el fitoplancton del Lago Morenito tuvo la mayor inhibición fotosintética acumulada (44 %), mientras que en los Lagos Moreno, Trébol, Nahuel Huapi y Gutiérrez la inhibición fue de 22, 11, 5 y 1 %, respectivamente. Sin embargo, al final de la incubación, y luego de recibir una dosis de 5 kJ m⁻², el fitoplancton más inhibido fue aquel del Lago Moreno (70 %), y el más resistente aquel del Lago Gutiérrez (27 %). La cinética de inhibición fue distinta para cada lago, registrándose una mayor velocidad en los lagos más transparentes y con mayor proporción de células grandes. Los resultados sugieren que, ante un aumento de UV-B (e.g., producto de una disminución de ozono estratosférico), el microplancton de lagos más claros será el más afectado mientras que el pico- y nanoplancton de lagos menos transparentes será el más resistente.

Palabras clave: radiación ultravioleta-B, fotosíntesis, fitoplancton, ozono, lagos andinos.

INTRODUCTION

Solar radiation is the principal energy source driving physiological processes in the majority of organisms. However, high levels of solar ultraviolet radiation, UVR (280-400 nm) may produce deleterious effects, such as those on fitness-related traits (e.g., motility, orientation) and impairment of physiological functions (e.g., photosynthesis and enzymatic reactions, Häder 1993, Holm-Hansen et al. 1993). Enhanced levels of solar UV-B (280-320 nm) have been detected due to the thinning of the stratospheric ozone layer (i.e., the ozone "hole", Farman et al. 1985) during the last three decades. Studies have demonstrated that ozone depletion events, associated with the Antarctic ozone "hole", also occur in mid to low latitudes of the planet (Kirchhoff et al. 1996, Tocho et al. 1996, Orce & Helbling 1997, Pérez et al. 1998), increasing the levels of UV-B that reach the Earth's surface. Both normal and enhanced levels of solar UV radiation are now considered to be a stress factor, and a variety of photobiological studies have been carried out to determine its effects and impacts on diverse organisms (Young et al. 1993, Caldwell et al. 1995, Figueroa et al. 1996).

The spectral characteristics and levels of UV radiation in water bodies depend on several factors. First, atmospheric and geographical factors will condition the amount of incident radiation reaching the water surface (Madronich 1993); second, the attenuation potential of a water body will be determined by the presence of particulate and dissolved (organic and inorganic) material (Kirk 1994); finally, physical processes (i.e., mixing) should be also considered to establish the radiation field at which the cells are exposed (Helbling et al. 1994). In particular, dissolved organic carbon (DOC) and mixing processes (produced by wind stress) seem to play a critical role in determining the underwater field of radiation in freshwater systems (Scully & Lean 1994, Morris et al. 1995). Andean lakes in the Patagonia region provide a variety of conditions in relation to their physical, optical, and biological characteristics (Diaz & Pedrozo 1993, 1996, Morris et al. 1995), therefore providing a very interesting scenario to carry out photobiological studies on phytoplanktonic organisms. In addition, the area is temporally influenced by the Antarctic polar vortex, thus affected by low-ozone air masses (Tocho et al. 1996, Orce & Helbling 1997, Pérez et al. 1998, Villafañe et al. 2001).

The aim of this paper was to determine the differential sensitivity to UV-B radiation (evaluated through the inhibition of photosynthesis) of

phytoplankton communities collected during July 1999, in five Andean lakes of the Patagonia region. These lakes cover a wide range of concentration of dissolved organic matter (DOM, Morris et al. 1995) that strongly condition the underwater field of radiation to which cells are exposed. These data will provide essential information about the mechanisms and potential for short-term acclimation to a sudden increase in UV-B due to the ozone depletion that would normally occur during the early austral spring.

MATERIAL AND METHODS

Surface water samples were taken daily early in the morning from five Andean lakes in the region of Bariloche, Río Negro, Argentina (41° S, 71° W, 800 m of altitude) by using an acid clean (1N HCl) carboy. The lakes sampled in this study (i.e., one each day) were Moreno, El Trébol, Gutiérrez, Morenito, and Nahuel Huapi. The lakes differed widely in their bio-optical characteristics (e.g., attenuation coefficients, amount of particulate and dissolved material), which provided a wide spectrum of potential responses of phytoplankton to UV radiation. Immediately after collection, samples were transferred to 50 ml acid clean (1N HCl) quartz tubes, inoculated with labeled sodium bicarbonate, and exposed to different doses of artificial radiation to determine photosynthetic rates (see below). Two radiation treatments were implemented: uncovered quartz tubes that received all radiation (UV + PAR) and quartz tubes covered with Mylar-D film that screened out all UV-B radiation such that samples received UV-A + PAR. Each radiation treatment had a total of eight replicate samples that were placed in an illuminated and temperature controlled chamber (at 10 °C), over a slow rotating plate (1 rpm) to ensure that all the tubes received a similar radiation field. The chamber had a Spectroline XX15-B fluorescent lamp (Spectronics Corporation) as a source for UV-B radiation and fluorescent lamps (Philips daylight) for PAR. Since both types of lamps also emit a portion of UV-A radiation, samples (at 55 cm from the radiation source) received irradiances of 0.35 W m⁻² for UV-B (280-320 nm), 1.1 W m⁻² for UV-A (320-400 nm), and 10.8 W m⁻² for PAR (400-700 nm). The spectral distribution of the UV-B lamp has been published elsewhere (Zagarese et al. 1997). Lamps were covered with acetate film in order to screen off any traces of UV-C radiation that the tubes might emit; the spectral transmittance of the acetate was checked and the filter replaced daily before each experiment. Two samples from each radiation treatment were removed and

analyzed for carbon incorporation every hour as described below.

Analysis and measurements

Photosynthetic rates were determined following the technique described in Holm-Hansen & Helbling (1995). Samples were inoculated with 5 μCi (0.185 MBq) of labeled sodium bicarbonate ($\text{NaH}^{14}\text{CO}_3$), and, after an incubation period of 1-4 h, they were filtered onto Whatman GF/F filters (25 mm). The filters were placed in 7 ml scintillation vials and then exposed to HCl fumes overnight. After drying the filter, a scintillation cocktail (Wallac Optiphase HiSafe 3) was added, and the activity measured using a liquid scintillation counter. Photosynthetic inhibition by UV-B radiation was determined by comparing the two treatments implemented as:

$$P_{\text{inh}} (\%) = ([P_{\text{My}} - P_{\text{Qz}}] / P_{\text{My}} \cdot 100),$$

where, P_{My} represents the carbon fixed in the PAR + UV-A treatment, and P_{Qz} the carbon fixed in the PAR + UVR treatments. The inhibition rate (kinetics of photosynthetic inhibition) was calculated from the slope of the regression between the cumulative inhibition versus dose using a linear fit.

The determination of chlorophyll-a (chl-a) concentration was done fluorometrically (Holm-Hansen et al. 1965) using a Turner Designs TD700 fluorometer that was calibrated using pure chlorophyll-a from *Anacystis nidulans* (Sigma C 6144). An aliquot of 100 ml of sample was filtered onto a Whatman GF/F glass fiber filter (25 mm) and the photosynthetic pigments extracted in absolute methanol for at least 1 h at 4 °C (Holm-Hansen & Riemann 1978). After centrifugation, the fluorescence of methanol extracts were measured before and after acidification (HCl 1N). The chl-a concentration in cells less than 20 μm in effective diameter (pico-nanoplankton) was obtained by pre-filtering 100 ml of sample through a 20 μm Nitex® mesh, and chl-a determined as mentioned before for total chl-a.

The amount of UV-absorbing compounds were determined by filtering 2-4 l of water onto a Whatman GF/F glass fiber filter (47 mm) and the particulate material on the filter extracted in absolute methanol for at least 1 h at 4 °C. After extraction, samples were centrifuged and the optical density of methanol extracts measured using a Hewlett-Packard HP 8453E spectrophotometer from 250 to 750 nm in a 1 cm quartz cuvette as described in Helbling et al. (1996).

Water samples were also taken and fixed with buffered formaline (final concentration in the sample 0.4 %) for floristic analysis. The identification and counting of specimens were made using a Leica DM IL inverted microscope (Utermöhl 1958). In addition, size distribution and mean cell area (μm^2) of phytoplankton species of each lake were determined by attaching a video camera (Philips LDH 0462/00) to the inverted microscope and using image analysis. For this latter measurements, an aliquot of 25 ml of sample was settled overnight; 10 fields were analyzed and at least 100 cells were measured.

Three different instruments (that were intercalibrated) were used to obtain information on radiation conditions at which the phytoplankton cells were exposed: (1) a GUV-511 spectroradiometer (Biospherical Instruments, Inc.), which had four narrow-band channels for UV radiation centered at 305, 320, 340, and 380 nm, and a broad band channel for PAR (400-700 nm). This sensor was permanently installed at the Laboratorio de Fotobiología (Centro Regional Universitario de Bariloche, Universidad del Comahue) and recorded solar radiation every minute. From these data, UV-B (280-320 nm) and UV-A (320-400 nm) were calculated by applying the algorithms of Orce & Helbling (1997); (2) an underwater ELDONET sensor (Real Time Computers Inc.) that had radiation channels for UV-B (280-315 nm), UV-A (315-400 nm) and PAR (400-700 nm), as well as channels for measurements of temperature and depth. This instrument was set to acquire one data per second, connected to a lap top computer, and it was slowly deployed by hand to determine the underwater radiation field in the studied lakes; (3) a portable IL1700 radiometer (International Light Inc.) that had sensors for UV-B (280-320 nm), UV-A (320-400 nm) and PAR (400-700 nm) was also used to measure the underwater radiation field. Sensors were attached to a rigid stick (2-m long) graduated every 10 cm and to a rope (graduated every 25 cm), and deployed by hand down to 10 m or less in shallow lakes. The IL1700 was interfaced to a lap top computer such that 10 measurements were automatically obtained at each graduated depth (i.e., every 10 cm in the upper 2 m of the water column and every 25 cm down below) for each channel. The irradiance received by cells in the illuminated chamber was determined using the portable IL1700, which was placed next to the experimental tubes. Although the Commission Internationale de l'Eclairage (CIE) defines UV-B as 280-315 nm, we preferred the range of 280-320 nm, not only because it has been widely used in ecological studies, but also because our Mylar-D filter cuts almost all radiation below 320 nm.

RESULTS AND DISCUSSION

During the austral winter, the amount of solar radiation reaching Patagonia is rather low (Fig. 1A). However, an increase in solar UV-B radiation, associated with ozone depletion events over Antarctica, has been noted during early spring over the Patagonia region (Villafañe et al. 2001). The energy ratio between two wavelengths, one affected (e.g., 305 nm) and one independent (e.g., 340 nm) of ozone concentrations is shown in Fig. 1B. A normal trend of increase in this ratio is expected as the summer solstice approaches; then this energy ratio will have the maximum value due to a lower solar zenith angle. However, there are some deviations from this trend, indicating a relative increase in the shorter UV-B radiation (Fig. 1B); during those days, ozone concentration decreased (as estimated from the E305 / E340 ratio, Orce & Helbling 1997). This relative increase in UV-B radiation (e.g., the ratio UV-B / UV-A had abrupt daily changes from 0.04 to 0.07 during late winter to early spring) constitutes a special environment for phytoplanktonic organisms that utilize solar radiation for photosynthesis and are at the base of the aquatic food web. In our experiments, however, UV-A radiation levels in the incubation chamber were lower than those reaching the Bariloche area during the studied period, resulting in a relatively high ratio of UV-B/UV-A radiation inside the chamber (see Material and Methods). One potential problem with such low levels of UV-A radiation comes from the fact that UV-A is involved in photoreactivation of damages produced by UV-B radiation. Although in this study we did not examine photoreactivation, a previous study (Zagarese et al. 1997), using the same incubation chamber and radiation sources, demonstrated that these UV-A levels were enough to cause photoreactivation. In the following

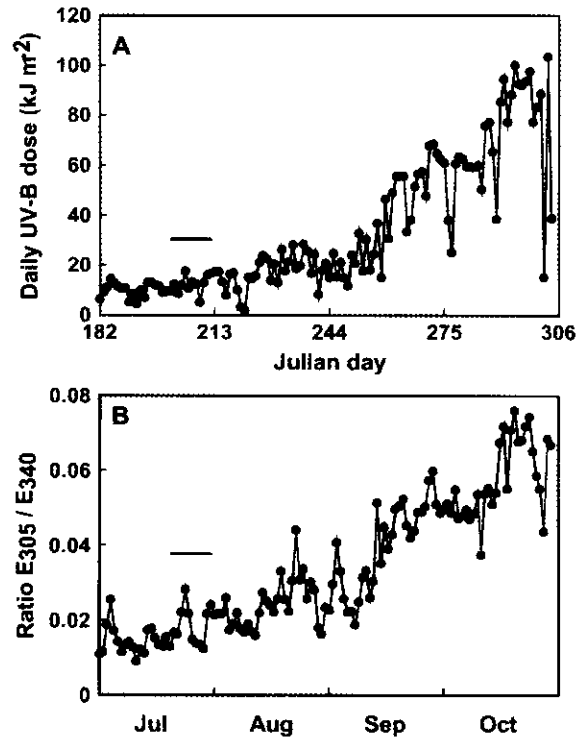


Fig. 1: Radiation conditions during mid winter-early spring (July 1 to October 31) of 1999 in the Bariloche area. (A) Daily dose of UV-B (in kJ m^{-2}) as a function of Julian day. The line over Julian days 202–210 indicates the study period. (B) Ratio of energies at 305 and 340 nm. The horizontal line indicates the study period. Data obtained with a spectroradiometer permanently installed at Universidad de Comahue in Bariloche.

Condiciones de radiación durante mediados de invierno-principio de primavera (1 de Julio al 31 de octubre) de 1999 para el área de Bariloche. (A) Dosis diarias de UV-B (en kJ m^{-2}) en función del día Juliano. La línea sobre los días Julianos 202–210 indica el período de estudio. (B) Relación de energías a 305 y 340 nm. La línea horizontal indica el período de estudio. Los datos se obtuvieron con un espectroradiómetro instalado permanentemente en la Universidad de Comahue en Bariloche.

TABLE 1

Bio-optical characteristics of five lakes studied in the Bariloche area. The attenuation coefficients were calculated using the data from an ELDONET radiometer

Características bio-ópticas de cinco lagos estudiados en el área de Bariloche. Los coeficientes de atenuación fueron calculados usando datos de un radiómetro ELDONET

Lake	Chlorophyll a ($\mu\text{g l}^{-1}$)		Attenuation coefficient (m^{-1})	
	Total	Cells < 20 μm	KUV-B	KPAR
Moreno	0.96	0.60	0.36	0.15
El Trébol	2.56	2.13	2.14	0.40
Gutiérrez	0.69	0.46	0.81	0.20
Morenito	1.32	1.11	2.80	0.46
Nahuel Huapi	1.38	0.79	0.42	0.12

paragraphs the impact of artificial, although realistic, UV-B radiation levels will be addressed for five Andean lakes with different underwater radiation field. The attenuation coefficients and biological characteristics (i.e., chl-a) of the five lakes are presented in Table 1.

Many studies (Karentz et al. 1991, Helbling et al. 1992, 1994, Schofield et al. 1995, Buma et al. 1997, Neale et al. 1998a, 1998b) have addressed the impact of UV radiation upon phytoplanktonic organisms and the influence some factors on this impact. The overall effect will be determined by the balance between damage/inhibition processes, and repair/acclimation capabilities (Lesser et al. 1994). Winter phytoplankton communities of Andean lakes are expected to be dark adapted not only due to the low irradiance levels observed (Fig. 1A), but also because of the occurrence of deep epilimnions resulting from thermal turnovers and strong wind patterns (Baigún & Marinone 1995). As a result of such light history, winter phytoplanktonic populations might experience a relative enhancement in their UV-B radiation field upon a sudden event of ozone depletion, as it usually takes place in late winter-early spring (Fig. 1B). One way to compare the sensitivity to UV radiation of phytoplankton from

various lakes is through exposing cells to the similar radiation sources and measure their photosynthetic rates. The ideal source of energy is solar radiation, but in this study, however, we were unable to expose phytoplankton from the five studied lakes simultaneously to the same natural radiation field. Instead, we used an artificial and constant radiation source, which also allowed us to obtain information on the kinetics of acclimation by means of taking samples at various intervals. Although we are aware of the problems when working with artificial sources of radiation, we used UV-B doses (i.e., 5 kJ m^{-2}) similar to those impinging upon the lakes at the moment of sampling (i.e., mean UV-B daily dose during the period of study was 11.6 kJ m^{-2}). A shift in the wavelength energy distribution will slightly affect the total value of cumulative inhibition. However, the rate of inhibition should remain more or less unchanged for each lake. We observed that, with the exception of phytoplankton samples from Lake Morenito, there was a steady inhibition of photosynthesis due to UV-B, which increased with dose (Fig. 2). After receiving an UV-B dose of 1.25 kJ m^{-2} (1 h of exposure), phytoplankton from Lake Morenito was the most affected (44 %), whereas samples from Lake Gutiérrez were the

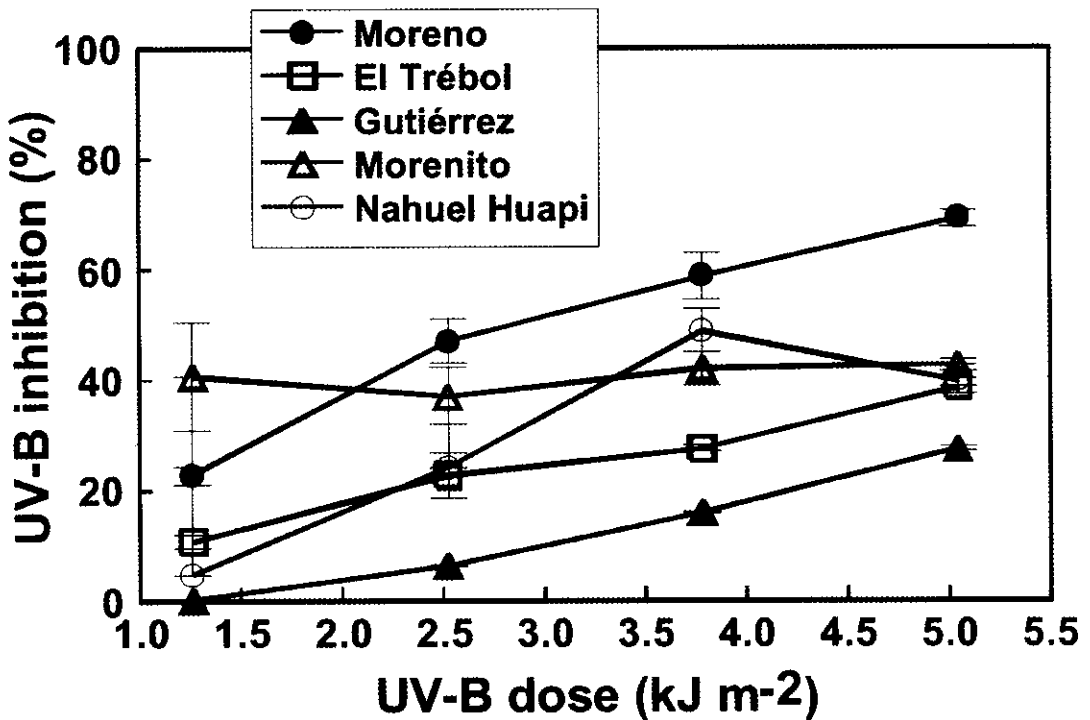


Fig. 2: Cumulative inhibition due to UV-B (%) as a function of the UV-B dose (in kJ m^{-2}) received by cells. Symbols represent different lakes as indicated in the inset. Vertical lines indicate one standard deviation.

Inhibición acumulada debida a UV-B (%) en función de la dosis de UV-B (en kJ m^{-2}) recibida por las células. Los símbolos representan diferentes lagos tal como se indica en el recuadro. Las líneas verticales indican una desviación estándar.

least (< 1 %). Phytoplankton from Lake Morenito, the least transparent lake for UV-B radiation (Table 1), apparently had a fast inhibition kinetics (i.e., less than 1 h), so the phytoplankton community was already inhibited by the first sampling (1 h). After that time, the cumulative inhibition increased slowly with time. Recent studies (EW Helbling unpublished results) have revealed a similar kinetics for phytoplankton from Lake Titicaca (Bolivia), in which, after an initial inhibition, the cumulative effect on photosynthesis remained almost constant with increasing irradiance/dose. The inhibition of photosynthesis for lakes Moreno, El Trébol and Nahuel Huapi after 1 h exposure, were 22, 11, and 5 %, respectively. However, at the end of the incubation period (4 h exposure), and after receiving a UV-B dose of 5 kJ m⁻², phytoplankton from Lake Moreno was the most inhibited by UV-B (70 %). In contrast, assemblages from Lake Gutiérrez were still the most resistant to UV-B radiation. There were significant differences ($P < 0.05$) in the cumulative inhibition of phytoplankton across lakes after 1 h exposure. At the end of the incubation period, however, there were no differences ($P > 0.1$) between lakes Morenito, El Trébol and Nahuel Huapi, but their cumulative inhibition were different from those of lakes Moreno and Gutierrez.

The sensitivity and kinetics of inhibition among the phytoplankton communities from the five stud-

ied lakes suggest differences in acclimation capability and/or mechanisms. One of the mechanisms whereby phytoplankton can acclimate to UV radiation is through the synthesis of UV-absorbing compounds such as mycosporine like aminoacids, MAAs (Dunlap et al. 1986, Shick et al. 1996). The duration of our experiments was short (4 h) to allow acclimation of phytoplankton through the synthesis of MAAs. However, the data obtained in this study (Fig. 3) suggest that this mechanism was not important in the field to these winter phytoplankton communities, as the chlorophyll-specific absorption indicated a similarly low concentration of MAAs in all lakes. Similar results have been reported for other freshwater communities, including those of Lake Titicaca (Villafañe et al. 1999). The low concentration of UV-absorbing compounds in phytoplankton communities could be due to several reasons. First, it has been demonstrated, for marine phytoplankton populations (Helbling et al. 1996, Neale et al. 1998a), that the synthesis of UV-absorbing compounds occurs in response to an irradiance stimulus, either UV or PAR. During winter, solar radiation fluxes over Patagonia are low, so phytoplankton might not need protection against UV radiation. Second, even if irradiance received by cells increases (e.g., by shallowing of the epilimnion, changes in transparency of the lake, enhanced levels of UV-B due to ozone depletion), dynamics

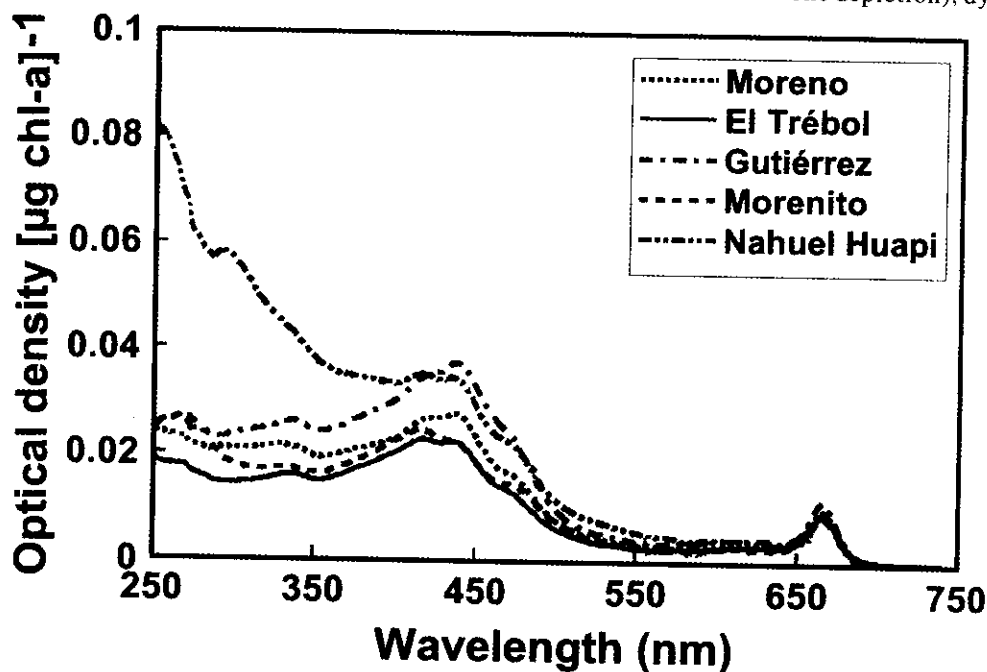


Fig. 3: Spectral characteristics of phytoplankton communities from five lakes. The ordinate is the optical density normalized to the concentration of chlorophyll-a, and the abscissa is the wavelength.

Características espectrales de las comunidades fitoplanctónicas de cinco lagos. La ordenada es la densidad óptica normalizada por la concentración de clorofila-a, mientras que la abscisa es la longitud de onda.

of UV-absorbing compounds could be too fast to be detected by accumulation in cells (Villafañe et al. 1999). Finally, Garcia-Pichel et al. (1994) demonstrated that the synthesis of UV-absorbing compounds in picoplanktonic cells ($< 2 \mu\text{m}$) is not an effective mechanism of protection against UV radiation, and that for the nanoplankton fraction (2-20 μm) such synthesis would represent a great energy loss at expenses of other processes (see also Sommaruga & Garcia-Pichel 1999). In many microplanktonic species, however, the synthesis of UV-absorbing compounds is an important mechanism for UV radiation protection (Helbling et al. 1996).

The size of phytoplankton cells influences the use of solar energy and other processes (e.g., turbulent motion, gas and nutrients exchange). Many studies have concentrated their scopes in the relationship between phytoplankton cell size and the effects of solar radiation (Karentz et al. 1991, Helbling et al. 1992, Laurion & Vincent 1998). Results are variable, but generally small cells (i.e., with a relatively high surface to volume ratio) are more resistant to photosynthesis inhibition, but more vulnerable to DNA damage (Helbling et al. 2001). On the other hand, large cells (i.e., microplankton) are more sensitive to UV radiation in terms of photosynthetic inhibition (providing they do not have high concentration of MAAs), but they are more resistant to DNA damage (Karentz et al. 1991, Helbling et al. 1992, 1994, Buma et al. 1997, Helbling et al. 2001). The phytoplankton community in our samples was mostly composed of cells smaller than 20 μm in effective diameter: between 55 % (Lakes Moreno and Nahuel Huapi) to 85 % (Lakes Morenito and El Trébol) of chl-a was in the pico to nanoplankton fraction (Table 1). Image analysis of phytoplankton samples also revealed the presence of pico-nanoplanktonic dominated assemblages, with a mean cell area ranging from 6 (Lake Morenito) to 33 μm^2 (Lake Moreno). Taxonomic analyses of samples also revealed the ubiquitous presence of small cells: monads and flagellates, mainly belonging to classes Chlorophyceae, Cryptophyceae and Crysophyceae; dinoflagellates were scarce in all samples; diatoms (Bacillariophyceae) were important in Lake Moreno, with only one species, the colonial *Aulacoseira granulata* (Ehr.) Ralfs that accounted for more than 50 % of the phytoplankton carbon biomass.

After plotting the cumulative inhibition as a function of community structure (based on cell size), we detected no clear relationship at any of the sampling intervals (not shown). Laurion & Vincent (1998), working with various size categories

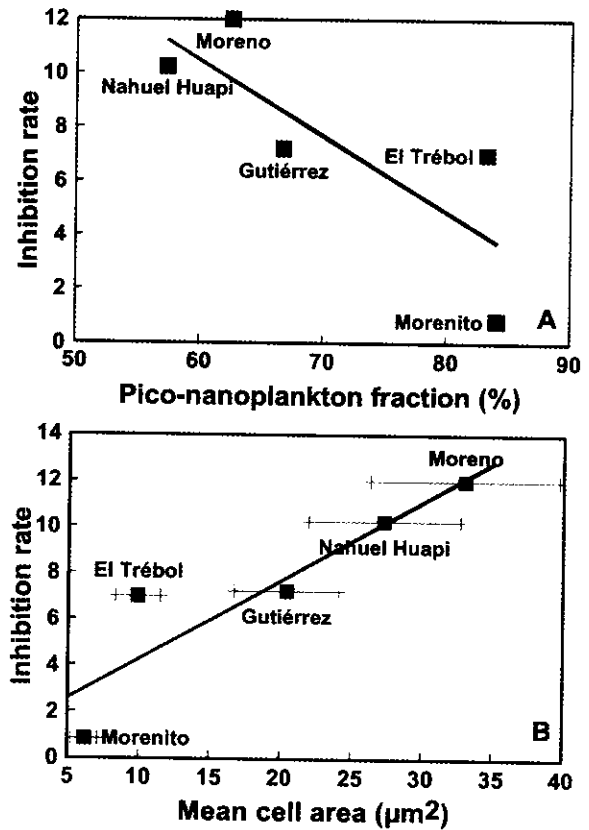


Fig. 4: Kinetics of photosynthetic inhibition as a function of the structure of phytoplankton community based on cell size, and as estimated by: (A) chlorophyll-a content (%) in the $< 20 \mu\text{m}$ cell fraction ($R^2 = 0.65$, $P = 0.099$), and (B) mean cell area (in μm^2) ($R^2 = 0.82$, $P = 0.032$). Solid lines indicate a linear regression fit. Horizontal lines indicate one standard deviation.

Cinética de inhibición fotosintética en función de la estructura de la comunidad fitoplanctónica basada en el tamaño celular, y estimada por: (A) contenido de clorofila-a (%) en la fracción celular $< 20 \mu\text{m}$ ($R^2 = 0,65$, $P = 0,099$) y, (B) área celular promedio (en μm^2) ($R^2 = 0,82$, $P = 0,032$). Las líneas llenas indican un ajuste por regresión lineal. Las líneas horizontales indican una desviación estándar.

ries of phytoplankton, have obtained similar results. However, when inhibition per unit dose (i.e., the slope from the linear fit of the curve in Fig. 2) were plotted against the community structure (i.e., cell size), a relationship was found (Fig. 4). Such a relationship ($R^2 = 0.65$) was inverse when inhibition rates were plotted against the chl-a content in cells $< 20 \mu\text{m}$ (Fig. 4A). Since the amount of chl-a in the different size fractions could vary (e.g., depending on the natural conditions at which the cells are exposed), we determined the mean cell area for each lake (see Material and Methods). A

plot between inhibition rate and mean cell area (Fig. 4B) revealed a positive relationship ($R^2 = 0.82$), with larger cells experiencing higher inhibition rates. The inhibition rate is a measure of not only how fast phytoplanktonic cells may be inhibited by UV-B, but also a measure of how resistant cells are. We are aware that cells may have acclimated to new condition of UV-B radiation over the 4-h experiments, but it should have been reflected in on inhibition rates (i.e., the rate would diminish). It has been demonstrated that the time scale for acclimation to UV radiation is closely linked to the process/target involved: the synthesis of UV-absorbing compounds has been shown to take from hours to days (Carreto et al. 1990, Helbling et al. 1996), whereas inhibition and acclimation processes involving photosystem II regularly take place in minutes (Schofield et al. 1995). This might be the case of lakes Moreno and Nahuel Huapi, where inhibition rates were high at the beginning of the experiment (Fig. 2), but decreased after certain dose was received (e.g., 2.5 and 3.75 kJ m^{-2} in lakes Moreno and Nahuel Huapi, respectively). Small cells seem to acclimate much faster as suggested by those of Lake Morenito that had the lowest, but significant, inhibition rate throughout the experiment.

When addressing the kinetics of photosynthetic inhibition it is important to consider the underwater radiation field at which the cells were exposed. This can be done by comparing the inhibition rate and the UV-B attenuation coefficients for each lake (Table 1), and as shown in Fig. 5. There was an inverse relationship between the kinetics of inhibition and the coefficient of attenuation ($R^2 = 0.80$). Lakes with low $K_{\text{UV-B}}$ had higher photosynthetic inhibition rates than lakes less transparent to UV radiation. Lakes El Trébol and Morenito were those with the highest attenuation coefficients (around 2-3 m^{-1} , Table 1, Fig. 5), the highest proportion of small cells (80-85 %, Fig. 4), and the lowest kinetic of inhibition (Fig. 4 and 5). Thus, it becomes clear that small cells, with a high surface to volume ratio, would be more fitted to utilize solar radiation in lakes with high attenuation of solar radiation. In these types of lakes mixing processes produce more variability as compared to relatively clear lakes. For example, if phytoplankton cells from Lake Morenito are moved vertically by 1 m they would experience an irradiance from three (KPAR based) to seven (KUV-B based) times higher than that of Lake Moreno. Metabolic processes of cells living in less transparent lakes need to be fast enough to

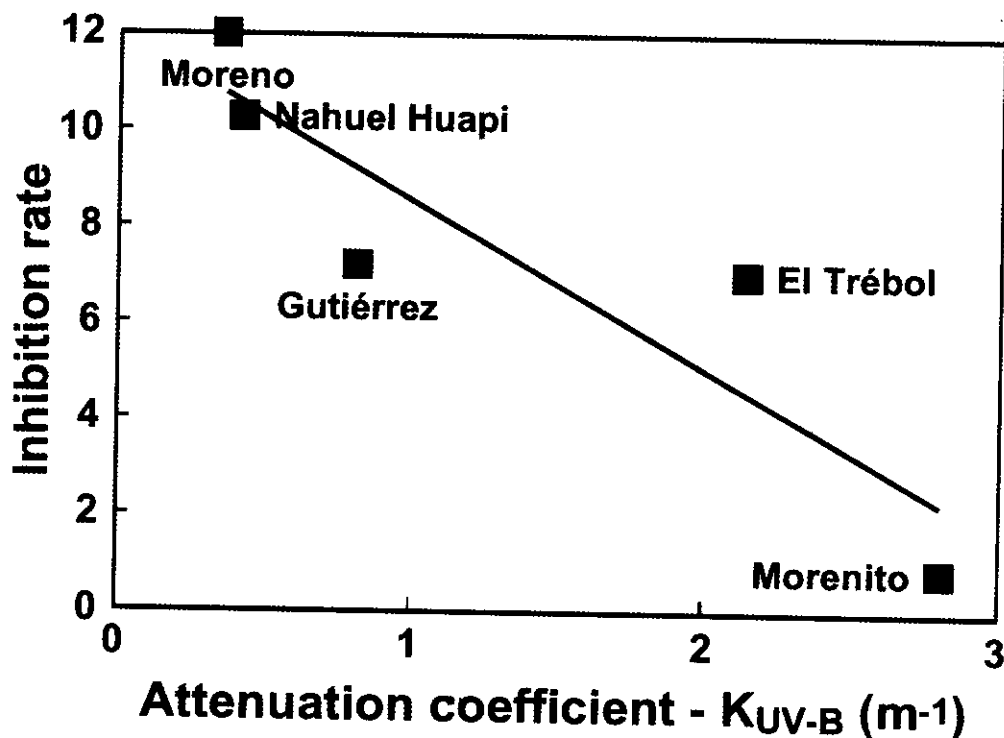


Fig. 5: Inhibition rate versus the attenuation coefficient for UV-B. The solid line indicates a linear regression ($R^2 = 0.80$, $P = 0.036$).

Tasa de inhibición en función del coeficiente de atenuación de UV-B. La línea llena indica una regresión lineal ($R^2 = 0.80$, $P = 0.036$).

accommodate such fluctuations of irradiance and then survive and grow in these changing environments.

Our results suggest that winter phytoplankton communities from clear lakes of the Andes region will be more vulnerable upon sudden changes in solar UV radiation, not only because of the high transparency of waters, but also because high inhibition rates characterize microplanktonic cells, common in these lakes. On the other hand, pico- and nanoplanktonic cells that dominate the biomass in less transparent lakes may have a faster acclimation capability to cope with environments of varying irradiance.

ACKNOWLEDGMENTS

We thank H. Zagarese for his help during the experiments and for providing UV radiation data. D.P. Häder loaned equipment and software for image analysis. L. Sala performed the ^{14}C analysis. We also thank Fundación Playa Unión for logistic support. This work was partially supported by the Agencia Nacional de Promoción Científica y Tecnológica -ANPCT/BID (grant PICT97 01-00046-00955), The Third World Academy of Sciences (TWAS 98-036/RG/BIO/LA) and by the Universidad Nacional del Comahue (grant B922). This is Contribution No. 15 of Estación de Fotobiología Playa Unión.

LITERATURE CITED

- BAIGUN C & MC MARINONE (1995) Cold-temperate lakes of South America: do they fit northern hemisphere models? *Archiv für Hydrobiologie* 135: 23-51.
- BUMA AGJ, AH ENGELEN & WWC GIESKES (1997) Wavelength-dependent induction of thymine dimers and growth rate reduction in the marine diatom *Cyclotella* sp. exposed to ultraviolet radiation. *Marine Ecology Progress Series* 153: 91-97.
- CALDWELL MM, AH TERAMURA, M TEVINI, JF BORNMAN, LO BJÖRN & G KULANDAIVELU (1995) Effects of increased solar ultraviolet radiation on terrestrial plants. *Ambio* 24: 166-173.
- CARRETO JI, MO CARIGNAN, G DALEO & SG DE MARCO (1990) Occurrence of mycosporine-like aminoacids in the red-tide dinoflagellate *Alexandrium excavatum*: UV-photoprotective compounds? *Journal of Plankton Research* 12: 909-921.
- DÍAZ MM & FL PEDROZO (1993) Seasonal succession of phytoplankton in a small Andean patagonian lake (Rep. Argentina) and some considerations about the PEG model. *Archiv für Hydrobiologie* 127: 167-184.
- DÍAZ MM & FL PEDROZO (1996) Nutrient limitation in Andean-Patagonian lakes at latitude 40-41° S. *Archiv für Hydrobiologie* 138: 123-143.
- DUNLAP WC, BE CHALKER & JK OLIVIER (1986) Bathymetric adaptations of reef - building corals at Davies Reef, Great Barrier Reef, Australia. III. UV-B absorbing compounds. *Journal of Experimental Marine Biology and Ecology* 104: 1-10.
- FARMAN JC, BG GARDINER & JD SHANKLIN (1985) Large losses of total ozone in Antarctica reveal seasonal ClOx/NOx interaction. *Nature* 315: 207-210.
- FIGUEROA FL, J JIMÉNEZ, JL PÉREZ-LLORENS & FX NIELL (eds) (1996) Underwater light and algal photobiology. Instituto de Ciencias del Mar, Barcelona, Spain. 343 pp.
- GARCÍA-PICHEL F (1994) A model for internal self-shading in planktonic organisms and its implications for the usefulness of ultraviolet sunscreens. *Limnology and Oceanography* 39: 1704-1717.
- HÄDER DP (1993) Risks of enhanced solar ultraviolet radiation for aquatic ecosystems. In: Round FE & DJ Chapman (eds) *Progress in phyecological research*: 1-45. Biopress Ltd., Bristol, United Kingdom.
- HELBLING EW, VE VILLAFANE, M FERRARIO & O HOLM-HANSEN (1992) Impact of natural ultraviolet radiation on rates of photosynthesis and on specific marine phytoplankton species. *Marine Ecology Progress Series* 80: 89-100.
- HELBLING EW, VE VILLAFANE & O HOLM-HANSEN (1994) Effects of ultraviolet radiation on Antarctic marine phytoplankton photosynthesis with particular attention to the influence of mixing. In: Weiler S & P Penhale (eds) *Ultraviolet radiation in Antarctica: measurements and biological effects*: 207-227. American Geophysical Union, Washington, District of Columbia.
- HELBLING EW, BE CHALKER, WC DUNLAP, O HOLM-HANSEN & VE VILLAFANE (1996) Photoacclimation of Antarctic marine diatoms to solar ultraviolet radiation. *Journal of Experimental Marine Biology and Ecology* 204: 85-101.
- HELBLING EW, AGJ BUMA, MK DE BOER & VE VILLAFANE (2001) In situ impact of solar ultraviolet radiation on photosynthesis and DNA in temperate marine phytoplankton. *Marine Ecology Progress Series* 211: 43-49.
- HOLM-HANSEN O & EW HELBLING (1995) Técnicas para la medición de la productividad primaria en el fitoplancton. In: Alveal K, ME Ferrario, EC Oliveira & EC Sar (eds) *Manual de métodos ficológicos*: 329-350. Ediciones de la Universidad de Concepción, Concepción, Chile.
- HOLM-HANSEN O & B RIEMANN (1978) Chlorophyll-a determination: improvements in methodology. *Oikos* 30: 438-447.
- HOLM-HANSEN O, D LUBIN & EW HELBLING (1993) Ultraviolet radiation and its effects on organisms in aquatic environments. In: Young AR, LO Björn, J Moan & W Nultsch (eds) *Environmental UV photobiology*: 379-425. Plenum Press, New York, New York.

- HOLM-HANSEN O, CJ LORENZEN, RW HOLMES & JDH STRICKLAND (1965) Fluorometric determination of chlorophyll. *Journal de Conseil pour l'Exploration de la Mer* 30: 3-15.
- KARENTZ D, JE CLEAVE & DL MITCHELL (1991) Cell survival characteristics and molecular responses of Antarctic phytoplankton to ultraviolet-B radiation. *Journal of Phycology* 27: 326-341.
- KIRCHHOFF VWJH, NJ SCHUCH, DK PINHEIRO & JM HARRIS (1996) Evidence for an ozone hole perturbation at 30° South. *Atmospheric Environment* 30: 1481-1488.
- KIRK JTO (1994) *Light and photosynthesis in aquatic ecosystems*. Cambridge University Press, Cambridge, United Kingdom. 509 pp.
- LAURION I & WF VINCENT (1998) Cell size versus taxonomic composition as determinants of UV-sensitivity in natural phytoplankton communities. *Limnology and Oceanography* 43: 1774-1779.
- LESSER MP, JJ CULLEN & PJ NEALE (1994) Carbon uptake in a marine diatom during acute exposure to ultraviolet B radiation: relative importance of damage and repair. *Journal of Phycology* 30: 183-192.
- MADRONICH S (1993) The atmosphere and UV-B radiation at ground level. In: Young AR, LO Björn, J Moan & W Nultsch (eds) *Environmental UV photobiology*: 1-39. Plenum Press, New York, New York.
- MORRIS DP, HE ZAGARESE, CE WILLIAMSON, EG BALSEIRO, BR HARGREAVES, B MODENUTTI, R MOELLER & C QUEIMALIÑOS (1995) The attenuation of solar UV radiation in lakes and the role of dissolved organic carbon. *Limnology and Oceanography* 40: 1381-1391.
- NEALE PJ, AT BANASZAK & CR JARRIEL (1998a) Ultraviolet sunscreens in *Gymnodinium sanguineum* (Dinophyceae): mycosporine-like amino acids protect against inhibition of photosynthesis. *Journal of Phycology* 34: 928-938.
- NEALE PJ, RF DAVIS & JJ CULLEN (1998b) Interactive effects of ozone depletion and vertical mixing on photosynthesis of Antarctic phytoplankton. *Nature* 392: 585-589.
- ORCE VL & EW HELBLING (1997). Latitudinal UVR-PAR measurements in Argentina: extent of the "ozone hole". *Global and Planetary Change* 15: 113-121.
- PÉREZ A, I AGUIRRE DE CARCER, JO TOCHO, E CRINO, HF RANEA SANDOVAL, ME BERNI, L DA SILVA, D HENRIQUES, F CUSSO & F JAQUE (1998) The extent of the ozone hole over South America during the spring of 1993, 1994 and 1995. *Journal of Physics D, Applied Physics* 31: 812-819.
- SCHOFIELD O, BMA KROON & BB PREZELIN (1995) Impact of ultraviolet-B radiation on photosystem II activity and its relationship to the inhibition of carbon fixation rates for Antarctic ice algae communities. *Journal of Phycology* 31: 703-715.
- SCULLY NM & DRS LEAN (1994) The attenuation of ultraviolet radiation in temperate lakes. *Archiv für Hydrobiologie, Beiheft See Ergebnisse der Limnologie* 43: 135-144.
- SHICK JM, MP LESSER & PL JOKIEL (1996) Effects of ultraviolet radiation on corals and other coral reef organisms. *Global Change Biology* 2: 527-545.
- SOMMARUGA R & F GARCIA-PICHEL (1999) UV-absorbing mycosporine-like compounds in planktonic and benthic organisms from a high-mountain lake. *Archiv für Hydrobiologie* 144: 255-269.
- TOCHO JO, L DA SILVA, G LIFANTE, F CUSSO & F JAQUE (1996) Penetración del agujero de ozono en Sudamérica. *Investigación y Ciencia* (febrero): 68-73.
- UTERMÖHL H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. *Internationale Vereinigung für Theoretische und Angewandte Limnologie* 9: 1-38.
- VILLAFANE VE, M ANDRADE, AV LAIRANA, F ZARATTI & EW HELBLING (1999) Inhibition of phytoplankton photosynthesis by solar ultraviolet radiation: studies in Lake Titicaca, Bolivia. *Freshwater Biology* 42: 215-224.
- VILLAFANE VE, EW HELBLING & HE ZAGARESE (2001) Solar ultraviolet radiation and its impact on aquatic ecosystems of Patagonia, South America. *Ambio* 30: 112-117.
- YOUNG AR, LO BJÖRN, J MOAN & W NULTSCH (eds) (1993) *Environmental UV photobiology*. Plenum Press, New York, New York. 479 pp.
- ZAGARESE HE, M FELDMAN & CE WILLIAMSON (1997) UV-B induced damage and photoreactivation in three species of *Boeckella* (Copepoda, Calanoida). *Journal of Plankton Research* 19: 357-367.